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10/772,996	02/05/2004	Shiv Kumar	PB0311	5385
7590 01/29/2007 Amersham Biosciences Corp 800 Centennial Avenue Piscataway, NJ 08855			EXAMINER BABIC, CHRISTOPHER M	
			ART UNIT	PAPER NUMBER
			1637	
SHORTENED STATUTORY PERIOD OF RESPONSE		MAIL DATE	DELIVERY MODE	
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**Please find below and/or attached an Office communication concerning this application or proceeding.**

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

<b>Office Action Summary</b>	<b>Application No.</b> 10/772,996	<b>Applicant(s)</b> KUMAR ET AL.	
	<b>Examiner</b> Christopher M. Babic	<b>Art Unit</b> 1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

#### Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

#### Status

- 1) ☒ Responsive to communication(s) filed on 19 October 2006.
- 2a) ☐ This action is **FINAL**.                      2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

#### Disposition of Claims

- 4) ☒ Claim(s) 1-12 is/are pending in the application.
- 4a) Of the above claim(s) 13-66 is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1-12 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

#### Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 05 February 2004 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

#### Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All    b) ☐ Some \*    c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

#### Attachment(s)

- |  |   |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)            | 4) <input type="checkbox"/> Interview Summary (PTO-413)           |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)   | Paper No(s)/Mail Date. _____                                      |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date <u>4/17/2006</u>   | 6) <input type="checkbox"/> Other: _____                          |

## **DETAILED ACTION**

### ***Election/Restrictions***

Applicant's election without traverse of group I, claims 1-12, in the reply filed on October 19, 2006 is acknowledged. Claims 13-66 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention(s) there being no allowable generic or linking claim.

### ***Claim Rejections - 35 USC § 112***

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

**Claim 4 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.**

Claim 4 is rejected as being indefinite for containing a Trademark(s) - Sequenase, Thermo Sequenase, NEB Vent, NEB Deep Vent, Novagen, Stratagene. If the trademark or trade name is used in a claim as a limitation to identify or describe a particular material or product, the claim does not comply with the requirements of the 35 U.S.C. 112, second paragraph. Ex parte Simpson, 218 USPQ 1020 (Bd. App. 1982).

The claim scope is uncertain since the trademark or trade name cannot be used

Art Unit: 1637

properly to identify any particular material or product. In fact, the value of a trademark would be lost to the extent that it became descriptive of a product, rather than used as an identification of a source or origin of a product. Thus, the use of a trademark or trade name in a claim to identify or describe a material or product would not only render a claim indefinite, but would also constitute an improper use of the trademark or trade name.

***Claim Rejections - 35 USC § 112 - New Matter***

The amendments to claims 5-7, filed October 19, 2006, encompass embodiments that were not contemplated or described in the application as originally filed. Specifically, the specification does not contemplate or describe utilization of the ligase enzyme within the claimed manganese concentrations. As discussed in the rejection under 112 1st paragraph below, claims 1 and 5-12 encompass a species of enzyme, ligase, that is not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the species. Thus, the amendment introduces the new concept of utilization of the ligase enzyme within the claimed manganese concentrations that was not contemplated or described in the application as originally filed.

This is a new matter rejection.

***Claim Rejections - 35 USC § 112 - Written Description***

Art Unit: 1637

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

**Claims 1 and 5-12 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.**

The written description requirement ensures that, "an applicant invented the subject matter which is claimed. Further, the written description requirement for a claimed genus may be satisfied *through a* sufficient description of a *representative number of species* by 1) reduction to practice; 2) reduction to drawing; or 3) disclosure of relevant identifying characteristics (*i.e.*, structure of other physical and/or chemical properties, functional characteristics *coupled* with a known or disclosed correlation between function and structure) (MPEP 2163 at II (A)(3)(a)(ii)).

In the instant case, claims 1 and 5-12 encompass a species of enzyme, ligase, that is not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the species.

Reduction to Practice

With regard to claims 1 and 5-12, although the specification discloses a genus including several species enzymes for use in the claimed method, the specification lacks the reduction to practice for the ligase enzyme with the claimed method. This is clearly evidenced by the lack of working examples as well as the use of the word, "ligase" in the specification. A cursory search of the specification found that the word, "ligase" was used only twice; once in the, "Description of Related Art" as well as the claims themselves. The specification references specifically on pg. 13 that different "polymerases" are used within the claimed invention, however, does not make reference to ligase. It is noted that the specification states that other suitable embodiments, "are not limited to" however it is submitted that one of ordinary skill in the art would not necessarily envision the enzyme, "ligase" as encompassed by the term, "polymerase" without specific mention given that the enzymes are routinely used in different context and have different structures as well as activities. Furthermore, the term, "polymerase" is routinely reserved within the art to describe enzymes that utilize single dNTP monomers in a "polymerization" reaction. Such evidence clearly demonstrates that there is no record or description which would demonstrate conception or description of using the enzyme ligase within the claimed invention.

Art Unit: 1637

Reduction to Drawing

The specification discloses several working examples of the claimed invention with enzymes such as DNA polymerase (figs. 1-8, for example), however, makes no reference to the use of ligase.

Disclosure of Relevant Identifying Characteristics

With regard to claims 1 and 5-12, while one could argue that a skilled artisan would be able to identify ligase enzymes that are demonstrate increased incorporation of terminal phosphated labeled nucleotides in the presence of manganese, however, such method would not satisfy the written description for the genus claims when, "the claims require an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art" (MPEP 2163(I)(A)). For the claims at hand, an example of an essential or critical feature is the chemical structure of the claimed enzymes. Applicants have not disclosed enough number of species of enzyme within the claimed genus that would indicate a characteristic chemical structure that would indicate that ligase would behave in the same fashion as, for example DNA polymerase to one of ordinary skill in the art.

Therefore, for the foregoing reasons, the genus embraced by the claims is not sufficiently described by the number of species disclosed in the specification, and therefore, the specification lacks written description of the claims.

***Claim Rejections - 35 USC § 112 – Scope of Enablement***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

**Claims 1-12 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods utilizing certain polymerases (i.e. Thermo Sequenase I DNA, Phi 29 exo-, AmpliTaq, TS I, Thy b, TS II, TS EM), does not reasonably provide enablement for methods utilizing all polymerases, ligases, telomerases, and primases.**

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

"Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in *Ex parte Forman*. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

**Scope of Enablement Issues**

This scope of enablement rejection is based on one fundamental enablement problem with the claims. This issue is a "how to use" problem in that the specification



Art Unit: 1637

does not reasonably enable one of ordinary skill in the art to practice the claimed invention utilizing all polymerases, ligases, telomerases, and primases, without undue experimentation due to the unpredictable nature of the invention. In other words, while the specification has reasonably provided evidence that manganese can significantly increase the ability of Thermo Sequenase I DNA, Phi 29 exo-, AmpliTaq, TS I, Thy b, TS II, and TS EM polymerase to incorporate terminal phosphate labeled nucleotides, the specification has not reasonably demonstrated that manganese will have the same effect on all polymerases, ligases, telomerases, and primases, as currently encompassed by the claimed invention.

#### The Nature of the Invention

The claims are drawn to a method of increasing the rate of an enzyme catalyzed nucleoside monophosphate transfer from a terminal-phosphate-labeled nucleoside polyphosphate. The invention is in the class of invention which the CAFC has characterized as "the unpredictable arts such as chemistry and biology." *Mycogen Plant Sci., Inc. v. Monsanto Co.*, 243 F.3d 1316, 1330 (Fed. Cir. 2001).

#### The Breadth of the Claims

The claims are broadly drawn to a method of increasing the rate of an enzyme catalyzed nucleoside monophosphate transfer from a terminal-phosphate-labeled nucleoside polyphosphate, wherein the enzyme can be *any* polymerase, ligase, telomerase, or primase. Thus, the claims encompass a broad range of enzymes (e.g. *all*

Art Unit: 1637

DNA polymerases, RNA polymerase etc.) from a broad range of species (e.g. human, bacteria, etc.).

#### Quantity of Experimentation

The quantity of experimentation in this area is immense since there is not only divergence within the claimed species of enzymes (e.g. polymerase and ligase), but divergence within the species of enzymes themselves (e.g. human RNA polymerase and E. coli RNA polymerase). In other words, not only do the overall chemical and biological characteristics of each species of enzyme vary, in some cases significantly, but they vary within each species as well. It would require significant study and experimentation to determine that manganese can significantly increase the ability of a particular enzyme to incorporate terminal phosphate labeled nucleotides. This would be an inventive, unpredictable, and difficult undertaking in itself, as any study including experimentation would need to be demonstrated with a statistically significant result. This would require inventive effort, with each of the many intervening steps, upon effective reduction to practice, not providing any guarantee of success in the succeeding steps.

#### The Unpredictability of the Art and the State of the Prior Art

The art suggests that it is entirely unpredictable how manganese will affect the ability of a certain enzyme to process a terminal phosphate-labeled nucleotide.

In a broader sense, the art suggests that it is entirely unpredictable how the

Art Unit: 1637

addition and/or substitution of manganese to a phosphoryl transferase reaction system will affect the kinetics of the enzymatic reaction. Schweins et al. ("The role of the metal ion in the p21ras catalysed GTP-hydrolysis:  $Mn^{2+}$  versus  $Mg^{2+}$ " J Mol Biol. 1997 Mar 7;266(4):847-56) highlights that:

"It has been shown for many enzymes involved in phosphoryl transfer, those which attack the  $\gamma$ -phosphate such as ATP/GTPases or the  $\alpha$ -phosphate such as **DNA polymerases**, but also for other enzyme catalysed reactions, that  $Mn^{2+}$  can substitute efficiently for  $Mg^{2+}$  in the active site. The replacement of  $Mg^{2+}$  by  $Mn^{2+}$  **has different effects on the kinetics of the enzymatic reaction in different systems**,"..., "In the case of inorganic phosphatase and DNA polymerase the rate is decreased in the presence of  $Mn^{2+}$ ,"..., "In the case of certain polymerases such as  $\theta$ 29 DNA polymerase  $Mn^{2+}$  has a different effect on the polymerase, 3'-5' exonuclease and priming activity,"..., "Thus, even though it has been shown or assumed, using evidence from many three-dimensional structures and from a number of EPR experiments with p21<sup>ras</sup> and other enzymes, that  $Mn^{2+}$  is a good substitute for  $Mg^{2+}$ , subtle differences in the properties of protein-substrate-metal ion complexes can lead to **drastic differences in the biochemical behaviour** (pg. 852, conclusion)."

Thus, it is clear from the overview of Schweins that one of ordinary skill in the art cannot accurately predict how  $Mn^{2+}$  will affect the biochemical properties of a phosphoryl transfer enzyme even within a species of enzyme (e.g. polymerase), much less between species (e.g. terminal transferase and polymerase). Furthermore, the complete substitution of  $Mg^{2+}$  by  $Mn^{2+}$  may even produce non-functional enzyme.

In addition, the findings of Hardin (U.S. 2003/0064366 A1) supplement an assertion of the unpredictable nature of the claimed invention by showing that different polymerases react differently to gamma-modified nucleotides (pg. 31, col. 1, for example). They specifically highlight that:

"Thus, for the Taq polymerase or the HIV1 reverse transcriptase, improved fidelity, due to the use of the gamma-modified dNTPs of this invention, enables single-molecule DNA sequencing. However, **not all polymerases equally utilize the gamma-**

**modified nucleotides of this invention**, specifically, Klenow, Sequenase, HIV-1 reverse transcriptase and Taq polymerases incorporate the modified nucleotides of this invention, while the **Pfu DNA polymerase does not appear to incorporate the modified nucleotides of this invention.**"

These findings are consistent with that of Tabor et al. (Effect of manganese ions on the incorporation of dideoxynucleotides by bacteriophage T7 DNA polymerase and Escherichia coli DNA polymerase I" Proc Natl Acad Sci U S A. 1989 Jun;86(11):4076-80) who found that T7 DNA polymerase and DNA polymerase I were not able to use a particular terminal phosphate-labeled nucleotide (deoxy-thymidine 5'-[ $\beta$ ,  $\gamma$ -methylene]triphosphate) in the presence of  $Mg^{2+}$  or  $Mn^{2+}$  (pg. 4078, col. 2; table 2, for example).

Thus, it is clear from the teachings of Hardin and Tabor that one of ordinary skill in the art cannot accurately predict whether a polymerase will even be able to utilize a terminal phosphate-labeled nucleotide, whether in the presence of  $Mg^{2+}$  or  $Mn^{2+}$ .

Thus, the combination of art teachings suggests that it is entirely unpredictable whether the addition and/or substitution of manganese to a polymerase, ligase, telomerase, or primase reaction system will increase the ability of such enzymes to incorporate terminal phosphate labeled nucleotides.

#### Working Examples

The specification discloses several working examples of the claimed invention utilizing Thermo Sequenase I DNA, Phi 29 exo-, AmpliTaq, TS I, Thy b, TS II, and TS EM polymerase (figs. 1-8, for example).

#### Guidance in the Specification

The specification provides no guidance or explanation as to how the demonstration of manganese to increase the ability of *one particular enzyme* to incorporate terminal phosphate labeled nucleotides suggests the same trend in another undisclosed "related" enzyme within a genus or even a species. For example, the specification provides evidence that the addition of manganese to a Phi29 *exo-* reaction system significantly increases the ability of Phi29 *exo-* to incorporate terminal phosphate-labeled nucleotides, however, provides no evidence that a significantly different untested polymerase enzyme such as *E. coli* DNA polymerase Klenow fragment (pol I K) (pg. 351, col. 1; Esteban et al. "Metal activation of synthetic and degradative activities of phi 29 DNA polymerase, a model enzyme for protein-primed DNA replication" Biochemistry. 1992 Jan 21;31(2):350-9) would behave in a similar manner.

#### Level of Skill in the Art

The level of skill in the art is deemed to be high.

#### Conclusion

In the instant case, as discussed above, the level of unpredictability in the art and the quantity of experimentation needed to establish that magnesium will increase the ability of a phosphoryl transferase enzyme to incorporate terminal phosphate labeled

Art Unit: 1637

nucleotides. The specification, while being enabling for methods utilizing certain polymerases (i.e. Thermo Sequenase I DNA, Phi 29 exo-, AmpliTaq, TS I, Thy b, TS II, TS EM), does not reasonably provide one with the written description or guidance that leads one to a reliable method utilizing any polymerase, ligase, telomerase, or primases. One of skill in the art cannot readily anticipate the effect of a change within the subject matter to which the claimed invention pertains. Further, the specification does not provide guidance to overcome art and specification recognized problems with the use of *any* phosphoryl transferase enzyme to incorporate terminal phosphate labeled nucleotides.

Thus, given the broad claims in an art whose nature is identified as unpredictable, the unpredictability of that art, the large quantity of research required to define these unpredictable variables, the lack of guidance provided in the specification, the presence of a working example which does not address the issue of the methylation in a broad range of disease states and the negative teachings in the prior art balanced only against the high skill level in the art, it is the position of the Examiner that it would require undue experimentation for one of skill in the art to perform all the claimed embodiments of the method as broadly written.

### ***Claim Rejections - 35 USC § 102***

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

Art Unit: 1637

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

**1. Claims 1, 3, and 5-7 are rejected under 35 U.S.C. 102(b) as being anticipated by Yarbrough et al. ("Synthesis and properties of fluorescent nucleotide substrates for DNA-dependent RNA polymerases" J Biol Chem. 1979 Dec 10;254(23):12069-73).**

With regard to claim 1, Yarbrough teaches a method (pg. 12072, col. 2, for example) comprising: a) conducting said enzyme catalyzed nucleoside monophosphate transfer from a terminal-phosphate-labeled nucleoside polyphosphate reaction in reaction buffer comprising a manganese salt (table III, for example); wherein said enzyme is selected from a template dependent nucleic acid polymerase (pg. 12072, col. 1, *E. coli* RNA polymerase, for example). It is noted that Yarbrough teaches a template independent reaction, however, the claimed method is anticipated because the claim does not require the presence of a template, and, *E. coli* RNA polymerase synthesizes RNA in the presence of a DNA template.

With specific regard to the phrase, "thereby increasing the rate of said reaction over the rate of said reaction in the absence of manganese" as included in claim 1, an increase in the rate of the reaction as compared to a reaction lacking manganese is a natural outcome of the methods taught by Yarbrough.

With regard to claim 3, Yarbrough teaches *E. coli* RNA polymerase (pg. 12072, col. 1, for example).

With regard to claims 5-7, Yarbrough teaches a manganese salt concentration of 2mM (table III, for example).

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

**Claims 1, 3, and 5-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wu et al. ("Synthesis and properties of adenosine-5'-triphospho-gamma-1-(5-sulfonic acid)naphthyl ethylamidate: a fluorescent nucleotide substrate for DNA-dependent RNA polymerase from Escherichia coli" Arch Biochem Biophys. 1986 May 1;246(2):564-71), Bernard et al. ("Synthesis of**



Art Unit: 1637

**complementary RNA on RNA templates using the DNA-dependent RNA polymerase of *Escherichia coli*" Biochim Biophys Acta. 1977 Oct 18;478(4):407-16), in further view of Yarbrough et al. ("Synthesis and properties of fluorescent nucleotide substrates for DNA-dependent RNA polymerases" J Biol Chem. 1979 Dec 10;254(23):12069-73).**

With regard to claim 1, Wu teaches a method (pg. 566, col. 1, total RNA synthesis, for example) comprising: a) conducting said enzyme catalyzed nucleoside monophosphate transfer from a terminal-phosphate-labeled nucleoside polyphosphate reaction in reaction buffer (pg. 566, col. 1, total RNA synthesis, for example); wherein said enzyme is selected from a template dependent nucleic acid polymerase (pg. 12072, col. 1, *E. coli* RNA polymerase, for example). It is noted that *E. coli* RNA polymerase synthesizes RNA in the presence of a DNA template.

With regard to claim 3, Wu teaches *E. coli* RNA polymerase (pg. 566, col. 1, total RNA synthesis, for example).

With regard to claims 8-10, Wu teaches a magnesium salt at 10mM (pg. 566, col. 1, total RNA synthesis, for example).

Wu does not expressly teach a reaction buffer comprising magnesium.

Bernard provides a supportive disclosure that teaches that *E. coli* RNA polymerase synthesizes RNA preferentially in the presence of magnesium ions (summary, fig. 4, for example). They expressly teach that RNA synthesis occurred at a

Art Unit: 1637

several fold higher rate in the presence of magnesium ions (pg. 410; fig. 4, for example).

With regard to claims 5-7, Bernard teaches a manganese salt concentration of between 0-10mM (table III, for example).

It is clear from the teachings of Bernard that one of ordinary skill in the art would have been motivated to add magnesium ions to the methods of Wu to increase the rate of nucleotide incorporation thus effectively increasing the incorporation of terminal-phosphate-labeled nucleotides, however, as discussed in the above enablement rejection, one of ordinary skill in the art, absent an express teaching, cannot accurately predict the biochemical behavior of a polymerase within the presence of magnesium and/or terminal phosphate-labeled nucleotides.

Though, in the case of *E. coli* RNA polymerase, it was well known that *E. coli* polymerase was able to incorporate terminal phosphate-labeled nucleotides within the presence of magnesium as demonstrated by Yarbrough as discussed above. In fact, Wu makes specific reference to the teachings of Yarbrough (pg. 565, co. 1, for example). Thus, there was a reasonable expectation of success at the time of invention.

Therefore, it would have been *prima facie obvious* to a practitioner of ordinary skill in the art at the time of invention to incorporate magnesium ions into the methods of Wu since Bernard and Yarbrough suggests such a modification to increase the rate of incorporation of nucleotides, thus arriving at the claimed invention.

**2. Claims 1, 3-7, 11, and 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hardin (U.S. 2003/0064366 A1) in view of McGuigan et al. ("DNA fingerprinting by sampled sequencing" Methods Enzymol. 1993;218:241-58).**

With regard to claim 1, Hardin teaches a method (pg. 31, col. 1, for example) comprising: a) conducting said enzyme catalyzed nucleoside monophosphate transfer from a terminal-phosphate-labeled nucleoside polyphosphate reaction in reaction buffer (pg. 31, col. 1, for example); wherein said enzyme is selected from a template dependent nucleic acid polymerase (pg. 31, col. 1, Sequenase, for example). Hardin further teaches gamma-phosphate labeled ddNTPs ([0244], for example).

Hardin does not expressly teach a reaction buffer comprising magnesium.

McGuigan provides a supporting disclosure that teaches that upon the addition of magnesium ions to a Sequenase reaction system, the polymerase incorporates ddNTPs at a greater rate (pg. 247, Enzymes, for example).

With regard to claim 3, McGuigan teaches Sequenase (pg. 12072, col. 1, for example).

With regard to claim 4, McGuigan teaches Sequenase (pg. 253, for example).

With regard to claims 5-7, McGuigan teaches a manganese salt concentration of 5mM (pgs. 251 and 253, 1X conc., for example).

With regard to claims 11 and 12, McGuigan teaches EDTA within the reaction mixture (pgs. 252 and 253, steps 9 and 10, for example).

It is clear from the teachings of Bernard that one of ordinary skill in the art would have been motivated to add magnesium ions to the methods of Hardin to increase the rate of ddNTP incorporation thus effectively increasing the incorporation of terminal-phosphate-labeled nucleotides, however, as discussed in the above enablement rejection, one of ordinary skill in the art, absent an express teaching, cannot accurately predict the biochemical behavior of a polymerase within the presence of magnesium and/or terminal phosphate-labeled nucleotides.

Though, in the case of Sequenase, it was known that Sequenase was able to incorporate terminal phosphate-labeled nucleotides as demonstrated by Hardin as discussed above. Thus, there was a reasonable expectation of success at the time of invention.

Therefore, it would have been *prima facie obvious* to a practitioner of ordinary skill in the art at the time of invention to incorporate magnesium ions into the methods of Hardin since McGuigan suggests such a modification to increase the rate of incorporation of ddNTP incorporation, thus arriving at the claimed invention.

### ***Conclusion***

**Claims 1-12 are rejected. No claims are allowed.**

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure:

Williams (U.S. 6,232,075). Williams teaches methods of polymerase incorporation of terminal phosphate-labeled nucleotides, however, does not teach these methods within the presence of manganese.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christopher M. Babic whose telephone number is 571-272-8507. The examiner can normally be reached on Monday-Friday 7:00AM to 4:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.


Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Application/Control Number: 10/772,996

Page 21

Art Unit: 1637

Christopher M. Babic  
Patent Examiner  
AU 1637

  
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PRIMARY EXAMINER

12/28/06